Posters

Protein Structure Prediction & Drug Design

3140-Pos Board B1

Integrative Structure Determination of the Components of the Nuclear Pore Complex by X-Ray Crystallography, Small Angle X-Ray Scattering, Electron Microscopy, NMR, and Comparative Modeling

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The Nuclear Pore Complex (NPC, ~50 MDa) is the sole passageway for the transport of macromolecules across the nuclear envelope. The NPC plays a key role in numerous critical cellular processes such as transcription, and many of its components are implicated in human diseases such as cancer. Previous work (ref 1, 2) defined the relative positions of its 456 constituent proteins (nucleoporin or Nups), based on spatial restraints derived from biophysical, electron microscopy, and proteomic data. Further elucidation of the evolutionary origin, transport mechanism, and assembly of the NPC will require higher resolution information. As part of an effort to improve upon the resolution and accuracy of the NPC structure, we set out to determine the atomic structures of the NPC components. Because it proved difficult to determine the atomic structures of whole Nups by X-ray crystallography alone, we are relying on multiple datasets that are combined computationally by our Integrative Modeling Platform (IMP) package (http://salilab.org/imp). In particular, we developed an integrative modeling approach that benefits from crystallographic structures of fragments of the protein or its homologs, Solution Small Angle X-ray Scattering (SAXS) profiles of the protein and its fragments (ref 3), NMR, and negative stain Electron Microscopy (EM) micrographs of the protein. Each dataset is converted into a set of spatial restraints on the protein structure, followed by finding a model that satisfies the restraints as well as possible using a Monte Carlo / molecular dynamics optimization procedure. The approach will be illustrated by its application to yeast Nup133.

1. Alber et al., Nature 450, 683-694 (2007).
2. Alber et al., Nature 450, 695-701 (2007).
3. Förster et al., J Mol Biol 382 (4), 1089-1106 (2008).

3141-Pos Board B2

A Next Step in Protein Secondary Structure Prediction

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We report on a new methodology for protein secondary structure prediction based on: step 1) constructing a new scoring function by taking short and long distance triplet residues interactions into consideration, 2) generating reference states from protein database with high similarity, and 3) using a genetic algorithm to refine the predictions from the consensus templates of existing secondary structure prediction methods to utilize both near and intermediate distance context. In most targets we tested, we found that our method at worst performs essentially as well as the best of the other constituent methods and at best performs much better. At the submission time of this abstract we believe that the performance limitations are lack of code optimization to fully utilize more compute power and do more exhaustive context dependent probability matrix for scoring. Our ultimate goal is to combine new improved secondary structure prediction methodology with improved loop protein structure prediction from our team (Rata et al, J Phys Chem B, 2010; Li et al, BMC Structural Biol. 2010; Li et al, JCIM, 2011) to enable improved tertiary structure prediction. Supported by NSF grant 1066471 to Li and NSF grant 489521 to Jakobsson.

3142-Pos Board B3

AWSEM-MD: Coarse-Grained Protein Structure Prediction using Physical Potentials and Bioinformatically Based Local Structure Biasing

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A coarse grained protein model was used for structure prediction. Prediction results are presented for varying degrees of local structure biasing based on a simple sequence alignment procedure to proteins with known structure. The local structure biasing is complemented by several physically motivated interactions. Pairwise direct contact and many body burial and water/protein mediated interactions were optimized using energy landscape theory. Alpha helical and beta strand hydrogen bonding potentials were parameterized using bioinformatic surveys. All of these potentials and several others, collectively known as the Associative memory Water mediated Structure and Energy Model (AWSEM), were recently integrated with LAMMPS, a popular open source molecular dynamics simulation package.

3143-Pos Board B4

Insights on the Evolutionary Conservation of Mating-Type HMG Domains are Revealed using Multiple Homology Models

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MATα1 works in coordination with MCM1 and other transcriptional regulatory proteins (ie. STE11) to activate transcription of α-specific genes and ultimately determine yeast cells mating type. Although structural knowledge exist for MCM1, MATα2 and MATa1, for MATα1 and all other mating-type homologues of MATα1, they are non-existent. Recent studies have suggested that the highly conserved alpha-domain of MATα1 belongs to the HMG family of DNA binding proteins. Analysis of 27 HMG domain structures in the Protein Data Bank allowed us to make theoretical predictions on the structure of the HMG domain of MATα1. A highly conserved α-helix required for DNA binding in all HMG domains and shares 50% homology to the structure of Lef-1/DNA. A base specific interaction using a conserved arginine is not seen in current HMG structures determined to date but is predicted in our models. Ultimately we hope that the model structures will yield further insight on the evolution of the HMG and the α-domain. Attempts to determine the structure of MATα_HMG domain by X-ray crystallography are currently being pursued and will also be discussed.

3144-Pos Board B5

Refinement and Quality Assessment of Predicted Protein Structures

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In recent years in silico protein structure prediction reached a level where a variety of servers can generate large pools of near-native structures. However, the identification and further refinement of the best structures from the pool of decoys continue to remain problematic. To address these issues we have developed the MUFOLD-MD server that uses the Rosetta software for structure refinement and a molecular dynamics (MD) based ranking (MDR) method for structure selection. The refinement of the selected structures is done by employing Rosetta's relax mode, subject to certain constraints. MDR selects the best structures by testing their relative stability against gradual heating during all atom MD simulations. The MUFOLD-MD server uses three sequential steps consisting of, i.e., structure: 1) generation, 2) refinement and 3) selection. 1) By using sequence-profile alignment (e.g., PSI-BLAST) and profile-profile alignment (e.g., HHSearch) methods, the query sequence is classified as either ''hard'' or ''easy'' target. For hard targets, models are generated using the Rosetta 3.2 software (ab initio method) and then ranked by using their Rosetta energy score. For easy targets, models are generated with the Multi-Dimensional Scaling (MDS) method and then ranked using the OPUS_Ca scoring function. 2) The structures (subject to certain constraints) are refined by employing the ''relax'' mode of Rosetta 3.2. 3) The MDR method is used to select the top 5 structures as the output of the server. Our MUFOLD-MD server was tested in both CASP8 and CASP9 competitions. Based on the official CAP8 results, MUFOLD-MD was ranked as number one server in the Free Modeling category.

3145-Pos Board B6

Physics Based Protein Structure Refinement

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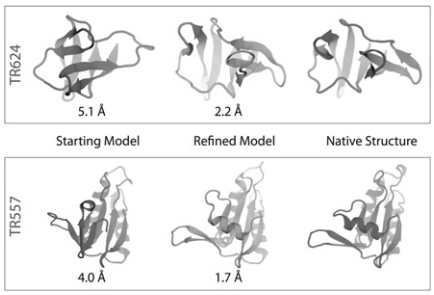
Accurate protein structure predictions are important for a number of purposes ranging from computational drug design, understanding experimental data and designing new experiments, to the emerging technique of de novo phasing in crystallography. The process of protein structure refinement occurs at the end of the structure prediction pipeline. The goal is to take an approximately correct starting model and further refine the details to produce a more accurate prediction. We have developed a physics-based approach to refinement that combines Hamiltonian exchange molecular dynamics with bioinformatics-derived restraints. The use of restraints dramatically reduces the volume of phase space that must be sampled and makes the procedure practical on small to medium

size proteins. We combine this approach with GPU-accelerated molecular dynamics, new implicit solvent models, and recent improvements in force fields. The initial results of this protocol are encouraging and we are able to successfully refine several difficult CASP9 refinement targets.



3146-Pos Board B7

New Developments on Generalized Simulated Annealing Applied to ab-initio Protein Structure Prediction

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Proteins are the building blocks of cells and the executioners of nearly all cellular functions. Their activity directly depends on their specific three dimensional structure, determined by the folding of its amino acid chain. The folding process ultimately creates a stable structure balancing internal contacts between amino acids and their occlusion to create the protein surface and the hydrophobic core. In this work we explore a new design for applying Generalized Simulated Annealing (GSA) on protein structure prediction, based on previous software developed by our group. The GSA is a stochastic search algorithm employed in energy minimization and used in global optimization problems, such as gravity models, fitting of numerical data and conformation optimization of small molecules. The software deploys a new way of updating the protein structure at each step of the simulation, a different potential energy calculation function based on NAMD and parallel execution of simulations, granting a new take on ab-initio protein structure prediction. The design of the software also allows for the inclusion of data derived from large scale analysis of protein structures from the PDB, as the Solvation Free Energy, allowing us to use information already gathered by experimental structure determinations. We present results on the 20 residue trp-cage mini protein and mastoparan-X, a 13 amino acid peptide. Both chains fold with RMSD of 0,2 nm and 0,1 nm respectively after 10000 GSA steps and a molecular dinamics optimization of 1 ms for trp-cage and 200 ns for mastoparan-X. Structure prediction softwares allow us to study protein structures that cannot be experimentally determined, by using data on chemical bonds, non-bonded interactions and protein solvation. Once approximately predicted, the three dimensional structure can be refined by molecular dynamics simulations.

3147-Pos Board B8

Structure-Energy Relationship of Biological Halogen Bonds: Development of Anisotropic Force Fields

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Halogen bonds (X-bonds) result from electrostatic attractive interaction between the electropositive crown of a polarized halogen, X, and an electron-rich Lewis base or accepting atom, A, resulting in an X_A distance closer than the sum of traditional van der Waals radii. X-bonds have been shown to direct protein ligand recognition and binding as well as the conformation of biological molecules. We have demonstrated via x-ray crystallography the ability of X-bonds to direct isomeric conformation of DNA Holliday Junctions. The stacked-X junctions can isomerize between two conformations; an X-isomer stabilized by X-bonding at the junction crossover, or the H-isomer stabilized by hydrogen bonding (H-bonding) at the junction crossover. The structures of DNA Holliday junctions incorporating fluorine (F), chlorine (Cl), bromine (Br), or iodine (I) halogenated uracil were determined by single crystal x-ray diffraction from 1.6 to 2.2 Å resolution. Stabilizing X-bonds between the halogenated uracil and phosphate oxygen demonstrate a near linear angle of approach of the oxygen towards the halogen, consistent with current halogen polarization and sigma hole theory. The ratio of each isomer observed in the crystal structure was determined via occupancy refinement calculations. We have shown that this ratio is correlated with the isomeric concentrations present in solution and therefore an indication of stabilization energy provided by either the X- or H-bonding, a conclusion supported by differential scanning calorimetry. We observe that halogen polarization affects both the X-bond structure and strength. The resulting structure and energy relationships of observed X-bonding interactions will be employed in development and parameterization of an anisotropic force field to accurately model the electrostatic and geometric treatment of halogens in current modeling programs. This will facilitate the applications of X-bonding interactions as a tool for biomolecular design and engineering.

3148-Pos Board B9

Absolute Binding Free Energy Calculations to Improve the Accuracy of Near-Native Ligand Pose Predictions

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When the high-resolution structure of a protein target is available, molecular docking experiments are commonly used for computer-aided drug discovery. Molecular docking experiments are advantageous since they can provide the binding mode of a molecule in a given target protein as well as the binding affinity. However, despite considerable efforts, accurate prediction of ligand poses bound to a target is still challenging due to the protein's structural flexibility. Recent free energy perturbation molecular dynamics simulation (FEP/MD) calculations have shown that the calculated binding free energies are in good agreement with experimental data for co-crystal benchmark targets. Here, we present an integrated methodology in which initial candidate selection from pose decoys obtained by docking is followed by FEP/MD calculations to improve the accuracy of near-native ligand pose prediction. Our approach is evaluated for the small molecule α-helix mimetics inhibiting protein-protein interactions such as p53-MDMX/MDM2 and BAK-MCL-1. The results demonstrate that using the centroid models of the most populated clusters of docking decoys is an efficient approach to select a small set of ligand conformations in which a near-native pose may be included and applying the FEP/MD method enhances ability in discriminating the near-native ligand conformation from the candidates.

3149-Pos Board B10

Structure Based Drug Design in Novel Druggable Pockets on Rho Family GTPases

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Rho GTPases are conformational switches that control a wide variety of signaling pathways critical for eukaryotic cell development and proliferation. They represent attractive targets for drug design as their aberrant function and up-regulation is associated with many human diseases including cancer. Extensive high-resolution structures and mutagenesis studies have laid the foundation for the design of new structure-based chemotherapeutic strategies. We describe the application of molecular dynamics (MD) simulations, principal component analysis, sequence conservation analysis, fragment mapping and ensemble small-molecule docking to provide a complete mapping of potential small-molecule binding pockets.

Characterized sites include novel pockets in the vicinity of conformationaly responsive switch regions as well as distal sites that appear to be allosterically linked to the nucleotide and effecter binding regions. Intensive virtual screening and docking calculations applied to these specific novel sites revealed promising compounds candidates for experimental assays. Furthermore, the use of a single accelerated MD simulation, advanced MD method that extends the accessible time-scale of conventional simulations, reveals a distribution of binding sites equivalent to the sum of the accumulated crystal structures including transient binding sites that are practically impossible to observe with conventional MD.

3150-Pos Board B11

Investigating the Mechanism of Activation and Inhibition of the Phosphate Dependent Mitochondrial Glutaminase

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Glutamine metabolism often plays a crucial role in the metabolism of cancer cells that exhibit high rates of aerobic glycolysis. The enzyme primarily responsible for the conversion of glutamine to glutamate, and ultimately the rate of α-ketoglutarate flux into the citric acid cycle, is the mitochondrial enzyme glutaminase. The fully processed form of the human enzyme can be expressed in bacteria, is fully active, and in this study we use a combination of